FINAL REPORT

OFFICE OF NAVAL RESEARCH PROJECT

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Contract: Nonr-803(00)

CONFIGURATIONAL CHANGES IN PROTEIN MOLECULES SUBJECTED TO VARIOUS CHEMICAL AND PHYSICAL STIMULI

CONTRACTOR: Iowa State College, Ames, Iowa

PRINCIPAL INVESTIGATOR: Joseph F. Foster

Introduction

The research program conducted, during the past two years, under this contract is a part of a very broad, long-term investigation of the physical-chemical behavior of proteins. In spite of the intensive study currently being devoted to this problem in a large number of laboratories all over the world, no ultimate solution can be anticipated for a great many years. In a sense, in view of the vital nature of the proteins, the problem is almost as big as that of life itself. It is felt that the results of the research program summarized in this report represent small, but none the less significant and important, footholds in the long ascent toward the peak which is our full understanding of the structure and behavior of proteins.

The broad objective of this program, as stated in the original research proposal, is "an application of some of the new physical-chemical techniques of polymer chemistry to a study of the changes in degree of nature of folding of the polypeptide chains in native globular proteins when subjected to various so-called denaturing conditions". The more specific objectives to be attacked were stated as:

(1) Extension of the streaming birefringence studies currently in progress on heat, urea and detergent denaturation of ovalbumin and serum albumin to other well characterized crystalline proteins and perhaps to some of the less well characterized proteins of more obvious biological significance...

(2) Study of the effect of other reagents, for example fatty acid anions, in promoting or inhibiting unfolding.

(3) Study of the effects of radiation on the state of folding of proteins, particularly ultraviolet radiation but possibly eventuallly even the higher energy radiations provided by the Iowa State College Synchrotron.

It was our opinion, initially, that during the course of a three year program, the period for which support was anticipated, objectives (1) and (2) could be largely attained and at least some beginning made on the third objective. Actually, the course of the investigation has not followed the plan indicated by these specific objectives. This is a consequence of the fact that, fairly early in the reaearch, an entirely unexpected observation with respect to the behavior of serum albumin in acid solution opened up a somewhat different approach. No apology is made for this; it is just another example of the fact that basic research can never be planned in advance.

This report terminates, at least for the time being, the formal relationships between the writer and O.N.R. He would like to take this opportunity to express his deep gratitude and appreciation to those many individuals associated with O.N.R. who have been so helpful in supporting and encouraging this work. These associations have been, from the writer's point of view, superb in every respect. He regrets that it was found necessary to terminate the contract earlier than anticipated. On the other hand, no serious difficulty has been caused since support for the continuation of the program has been obtained from the National Cancer Institute (U. S. Public Health Service). The

research will be continued in the Department of Chemistry of Purdue University, which Department he is joining on July 1, 1954.

Summary of Results Previously Reported (July 1, 1952 - Dec. 31 1953)

The first phase of the program was directed toward the completion of studies begun under previous sponsorship (Swift and Company and Rockefeller Foundation) on the binding of surface active ions by proteins and the relationship to protein denaturation. The two proteins studied, ovalbumin (O) and bovine serum albumin (BSA), were found to behave very differently. Further, important differences were observed in the mode of action of anionic as compared to cationic agents. No elaboration on these results appears warranted here since they have been described in two publications cited under Bibliography (1,2).

As an outgrowth of this research, an important observation was made with respect to the behavior of some solutions of surface active ions upon dialysis. This observation was followed up to the extent that a new method for determination of critical micelle concentrations was developed and published (3).

A further important growth of the studies with cationic detergents was the demonstration that BSA is subject to marked structural alterations when the pH of a solution is reduced from the isoelectric point to about 2 or 3. The second phase of the study, reported in a progress report dated January 12, 1954, consisted of a rather detailed exploration of this phenomenon using optical rotation, intrinsic viscosity, electrophoresis and streaming birefringence. This phase of the work has now

been published (4). The structural alteration was concluded to be a molecular swelling, induced by coulombic repulsion of the positive charges arising on the protein molecule at low pH. In addition to the pH effect, the effect of ionic strength was examined. It was postulated that the swelling is not a gradual one, but rather consisted of an all-or-none transition from the native (compact) form to a highly expanded form. The evidence for this postulation rests on the close one-to-one correspondence between the shifts in the two properties intrinsic viscosity and optical rotation, which properties were found to be particularly useful for study of the phenomenon. Electrophoresis and streaming birefringence were found to be, on the other hand, of relatively little utility in this particular study. It is for this reason that the shift in emphasis in the program, mentioned under Introduction, was adopted.

Progress Since Last Progress Report (January 1, 1954).

The studies of the acid-swelling phenomenon conducted during the last six months can be summarized under four general headings.

(1) Effect of temperature - It was considered important to ascertain the effect of temperature on the swelling equilibrium in the hope that the thermodynamic properties might be evaluated. It was soon observed that above 50°C a secondary, irreversible, transition takes place in acid solution. This transition is characterized by a marked decrease in the intrinsic viscosity of the solutions of the originally swollen protein

molecule with little or no attendant change in optical rotation. The process is not kinetically first order and the rate increases somewhat, but not drastically, with decreasing pH. The ultimate intrinsic viscosity attained is only about two or three times that of the native protein, in contrast to a maximal increase of about 20 times in the case of the swollen protein at zero time.

The irreversible reaction does not seriously complicate the study of the effect of temperature on the equilibrium, because of the slowness of the reaction (half-life of the order of several hours). Another complication arose, however, which does complicate the interpretation and which in fact almost negates the possibility of thermodynamic interpretation, at least on the basis of the simple process assumed. This complication arises from the fact that while above 20° the temperature dependence is positive, below that temperature down to 0° the properties appear to be almost temperature independent. This problem has not been resolved and will necessitate considerable future research.

(2) Effect of Urea - Published results by Kauzmann et al on the effect of urea on the optical rotation and intrinsic viscosity of BSA at pH near 7 prompted studies as to the effect of combined action of urea and acid. Studies have been carried out over the pH range 5 to 2, at three ionic strengths, in the presence of 2.0 molar urea. The results are in substantial qualitative agreement with those in absence of urea, but the swelling reaction is shifted toward higher pH values as might

be expected. The tentative interpretation is that the urea, by virtue of its ability to break intramolecular hydrogen bonds within the protein molecule, weakens the structure and permits expansion at lower net charge. It should be pointed out that in Kauzmanns studies no swelling was observed in 2 molar urea at the pH he used. One unfortunate feature of these studies is the fact that some irreversible alteration of the protein appears to take place, even at room temperature, in the presence of urea and acid.

(3) Effect of Dioxane - If the general conclusion, previously reached, that swelling is due to coulombic repulsion be correct, the dielectric constant of the solvent should be an important parameter. To investigate this point studies of optical rotation and intrinsic viscosity were carried out over the pH range 5 to 2 in presence of 10% and 20% aqueous dioxane. The results were qualitatively as expected. At 20% dioxane, however, some difficulty was encountered from irreversible insolubilization of a portion of the protein. The results at 10% dioxane, while experimentally satisfactory, do not permit extensive theorization because the alteration in dielectric constant is not sufficient (70 as compared to 79 for water).

(4) Extension of studies to other proteins - The observation that BSA and O behave entirely differently with respect to acid, O being essentially rigid and unexpandable, pointed up the importance of surveying as many other proteins as possible. The following proteins have been screened in a preliminary test for increases in the two properties, optical rotation and intrinsic viscosity, in acid solution: serum γ-globulin, β-lactoglobulin,

lysozyme, pepsin, trypsin, trypsinogen, chymotrypsin, chymotrypsinogen, and insulin. Of these proteins, the only one to show an appreciable effect was γ-globulin. These results suggest first of all that the behavior of BSA may be the exception rather than the rule (in other regards, too, notably with respect to its ability to bind small ions, BSA is rather exceptional.) The negative results with the enzymes are of interest in light of a theory of the mode of action of enzymes, suggested by Laidler, based on the idea that enzymes can reversibly alter their configuration during enzymic activation. On the other hand, the fact that γ-globulin, which is presumed to contain most if not all of the serum antibodies, expands readily raises many interesting possibilities with respect to antibody structure, function and atability.

At least three, and possibly four, publications are planned covering these results. It is hoped that these manuscripts may be written during the summer. They will, of course, be submitted to ONR at the time they are ready for publication.

In addition to these experimental studies, considerable time has been spent by the writer, during this period, in compiling and attempting to correlate in publishable form a large mass of data on the streaming birefringence behavior of denatured BSA. This has resulted in a manuscript which has been forwarded on to ONR and submitted to the Journal of the American Chemical Society.

Personnel -

The following personnel have been engaged on this project during the past six months:

J. F. Foster, Leader in Charge

Melvin Sterman, Research Associate (Half-time)

Miss Ana Pellecer, Technician (Hourly basis, approximately one-third time).

Dr. Jen Tsi Yang, who was employed full-time on the project last year, has been employed full-time on an entirely separate project in this laboratory during this last year but has been able to participate in a vital way, without monetary renumeration, largely through his direction of the efforts of the technician, Miss Pellecer.

Bibliography

(1) J.T. Yang and J. F. Foster. Statistical and All-or-none Binding of Alkylbenzenefulfonate by Albumins. J. Am. Chem. Soc. 75, 5560 (1953).

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(3) J. T. Yang and J. F. Foster. Determination of Critical Micelle Concentration by Equilibrium Dialysis. J. Physical Chem. 57, 628 (1953).

(4) J. T. Yang and J. F. Foster. Changes in the Intrinsic Viscosity and Optical Rotation of Bovine Plasma Albumin Associated with Acid Binding. J. Am. Chem. Soc. 76, 1588 (1954).

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